

Declaration of Co-inventor Yury Zelechonok
Traversing Rejection under 37 CFR 1.132

The undersigned, being warned that making willful false statements may jeopardize the validity of any patent obtained from this application, herein declares that:

1. He received in 1985 a PhD from the Institute of Element-Organic Compounds of the Academy of Sciences of USSR, Moscow, Russia with emphases on organic synthetic chemistry.

2. Since then, he has worked extensively in the field of liquid chromatography (LC) applied to organic synthesis, including:

(a) a 1990 to 1993 period as a Post Doc. at Northwestern University, Evanston, IL, working in the field of enzymology and drug design where LC was a main tool of analysis; and

(b) a 1993 to 2003 period at G.D. Searle (which later became Pfizer Company) at Skokie, IL, as head of an analytical support group that provided expertise and services to the entire company of liquid separation mainly by means of LC; and

3. During these mentioned periods: he personally handled and/or supervised directly in the development and separation of thousands of LC analysis for internal customers; he authored or co-authored more than 30 publications covering the field; he was the sole inventor or co-inventor of 5 patents related to the field; he regularly attended and participated in annual and other specialized LC seminars and conferences; he was familiar, via direct examination or personal communication, sales and technical pamphlets, and Internet and other modes of information transfer, with the LC columns available from most if not every U. S. and international manufacturer in continued attempts to improve on and/or solve specific LC analysis situations; and he personally, or indirectly under his direct supervision, used

many of these different LC columns in actual analysis situations.

4. In 2003, Pfizer closed the Skokie facility, whereby facing relocation but deciding to remain in the area, he took his unused vacation and severance benefits and started, with co-inventor Orlovsky (also having extensive work experience in LC analysis and having a Master of Science degree in chemistry), a technically driven start-up company originally named Allsep but later renamed to SIELC, located in Prospect Heights, IL; to wit:

(a) He and the co-inventor, with their accumulated and specialized skills and backgrounds, conceptualized a corporate purpose of developing and commercializing a LC product that it could by itself design, make and sell to a ready large field of users, where if the technical potential of the product was achieved and provided superior operational performance, it maybe even would be sought out by LC customers; and

(b) they selected LC columns, which were highly technical, widely needed, quickly expendable for repeat purchases, costly, and most importantly needed and used by people typically in almost any economy, particularly with the expectation that its column design could offer technical improvements that would overcome shortcomings existing in the LC column design.

5. The SIELC company efforts lead to the conception and development of this invention, that utilizes two commonly used functional groups (ion bearing and hydrophobic) uniquely combined and arranged to provide a new and vastly improved stationary phase, that could form a LC column that would overcome problems experienced with the old and long used existing LC technology, and further would achieve increased versatility of uses with improved and consistent results of analysis.

6. As a co-inventor of this invention, he is familiar with the now pending claims 9 and 10 which call for:

(a) an improved stationary phase for high performance liquid chromatography analysis of small molecules using a mobile phase, that comprises

a rigid supporting material having a surface;

an ion bearing functional group and a hydrophobic functional group chemically attached together end-to-end;

said ion bearing functional group being chemically attached directly to the rigid supporting material and said hydrophobic functional group being indirectly connected relative to the rigid supporting material surface via its connection to the ion bearing functional group; and

said hydrophobic functional group being remotely spaced from the rigid supporting material surface and overlying the ion bearing functional group and forming a permeable stationary layer shielding said ion bearing functional group from full contact with the passing mobile phase, and having a carbon chain at least eight carbon atoms long.

(b) he believes that prior to this invention, no LC column was commercially available that utilized the recited stationary phase; and

(c) the SIELC company has only made and sold columns utilizing the recited stationary phase.

7. Technically speaking:

a) the liquid phase separation of small molecules primarily is based on four main techniques of separation: normal phase, reverse phase, ion-exchange and ion-exclusion, respectively illustrated schematically in Figs. 1 to 4 of the application, each developed independently over the last 40 or so years;

(b) prior to this invention, each technique of separation required a specific column design and a specific mobile phase, and limitations could arise because of the coexistence of these four independent techniques;

1. several types of column would be needed to perform analysis for different classes of compounds;

2. compounds with dual properties (etc. ionic and polar or ionic and hydrophobic) could be analyzed only by one type of separation, which would limit the selectivity of the analysis;

3. each technique would not be applicable to complex mixtures comprised of different classes of compounds.

(c) This stationary phase invention overcomes these problems, such as:

1. each structured column can be used for the different techniques of normal phase, reverse phase, ion-exchange and ion-exclusion, due to the dual nature of the inventive stationary phase, and further the dual interactive mechanism can be provided by changing the mobile phase to alter the degree of each interaction, so that in fact every column can have or operate in or with different or multiple properties;

2. a compound with dual properties can be separated more efficiently with high selectivity by providing for simultaneous dual interaction;

3. polar-ionic and neutral-hydrophobic compounds can be analyzed simultaneously due to the ability of this new stationary phase to produce more than one type of interaction at the same time;

4. material similar to that described by Talley is commercially available, and Fig. 9 in the subject application shows a poor chromatogram obtained when using this material for separating a mixture of carboxylic acids, while an assignee column

made with material according to the current claim, with the same mobile phase mixture, produced the desirable widely separated component peaks of Fig. 7;

5. figs. 11 and 12 of this application comparatively illustrate separation of charged polar molecules (nucleosides), where Fig. 11 illustrates successful separation using the combined ion exchange and RP mechanisms material of the inventive column and Fig. 12 illustrates an unsuccessful separation using only the stationary phase materials of O'Gara.

7. Commercial Success of Invention, to wit:

(a) the SIELC company, the assignee of this application, started small, and even now has only the two co-inventors and three others as full-time employees, operating out of its office/plant/headquarters in Prospect Heights, Illinois;

(b) the SIELC company now makes and markets a broad selection of different columns utilizing the inventive stationary phase: having nine variations of the stationary phase, differing in the hydrophobic element and/or in the ionic charge; having six different column lengths, as short as 10mm and as long as 250mm; and having seven different diameters of the column, as small as 1mm and as large as 50mm;

(c) details of the inventive LC column constructions can be viewed by the Examiner in the SIELC company pending patent application No. 10/797,587 filed 03/11/04;

(d) sales are generated by approximately a dozen independent commission distributors located here in the States, in Canada and abroad; or by direct company to company contacts that may have arisen independently of any distributor, in part because the undersigned actively attends LC related seminars, conferences, and LC user's offices, throughout the world, to make educational or technical presentations of LC products and procedures, and to promote the inventive LC columns, responsive

to technical publications (see Appendix A for samples) the company has published and distributed to the distributors and interested or potential LC users, or via the Internet at the company web site at www.sielc.com.

(e) the SIELC company sales of columns utilizing the invention have increased almost expeditiously, from approximately \$50K in 2003, almost \$200K in 2004, and about double that to approximately \$400K in 2005.

8. The undersigned's opinion of Obviousness is that:

(a) the undersigned believes that if the invention were obvious, as the rejection holds, other competing manufacturers of LC products would have introduced such columns prior to the SIELC company; particularly in view of the increases and successes from column sales, considering also it's the only company product and the company started from nothing except for its most inventive column design;

(b) the SIELC company is now aware of one such old-time competitor (Dionex) that only recently is entering the market with what could be a copy-cat column (see the accompanying company flyer (Appendix B, page ^B27 which closely parallels the SIELC technical publication of August 2004, Appendix ^{A-18} A), where such copying is strong evidence that the competitor appreciates and is responding to:

(1) the operational superiority of SIELC's inventive column to its columns;

(2) its possible sales loss to the SIELC company column and its need to come out with its own version of or a copy-cat column; and

(3) the fact this product entry is only now, and not before the SIELC column design had been introduced, is strong evidence that the invention was not obvious to it;

(c) while they are not in declaration form or have the same viable impact, consider some recent comments (see Appendix C) directed to the LC chat-room of pleased or even amazed LC users of the inventive SIELC columns, which comments were made freely and without any prior request by the SIELC company and which show their acknowledgement of how effectively the inventive column operated and advanced the old field of LC; again suggesting that the inventive column was most significant and not obvious.

9. The undersigned herein declares that all statements made of his knowledge are true and all other statements made are believed to be true;

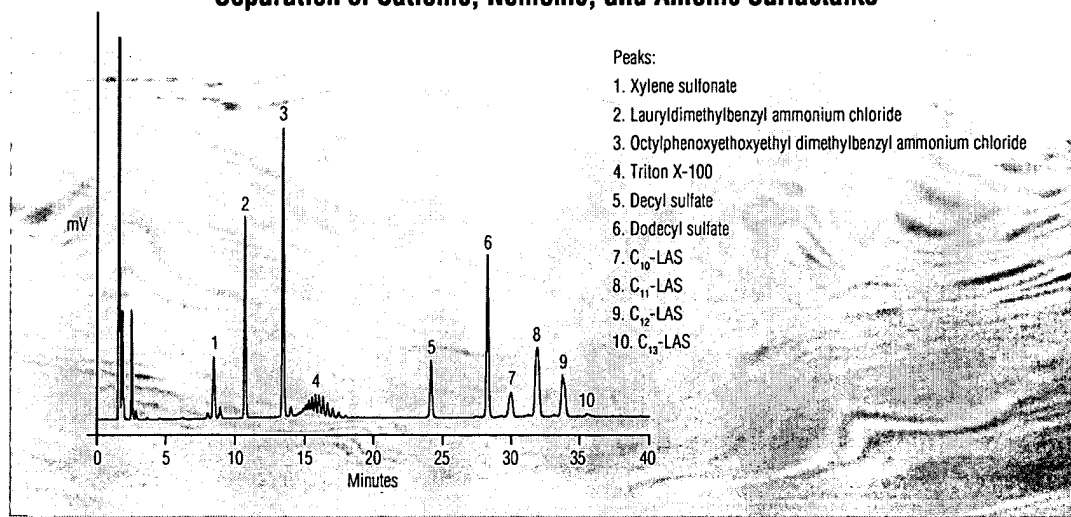

Yuri Zelechonok

Dated 02-04-06

Surfactant Column

Acclaim[®] Surfactant Column A Simple Solution to Difficult Challenges

Separation of Cationic, Nonionic, and Anionic Surfactants



Acclaim Surfactant columns are high-efficiency specialty silica columns for separating anionic, nonionic, and cationic surfactants:

- Ideal selectivity for separation of anionic, nonionic, and cationic surfactants
- Excellent peak shapes for cationic surfactants
- Improved resolution for ethoxylated surfactants
- Compatible with highly aqueous mobile phases
- Methods compatible with various detectors
- Broad range of applications

Ideal Selectivity for the Separation of Anionic, Nonionic, and Cationic Surfactants

The Acclaim Surfactant column is a new column designed for, and ideally suited to, the separation of a variety of different surfactants. This column incorporates a proprietary silica-based bonded phase that offers ideal selectivity and unprecedented capacity for separating cationic, nonionic and anionic surfactants in a single run. The simple, volatile mobile phases are compatible, with mass spectrometry detection which

facilitates the application of this column to trace-level analyses of surfactants in various matrices, including pharmaceutical formulations and environmental samples.

Surfactants are widely used in industrial, agricultural, and pharmaceutical markets, in products as diverse as pesticides, detergent powders, petroleum products, cosmetics, and pharmaceuticals. Their separation and identification can be a challenge due both to the diversity of surfactants and complexity of the sample matrix.



The separation of surfactants is typically accomplished using HPLC. Reversed-phase and ion-exchange chromatography are the most popular approaches, but normal-phase and size-exclusion chromatography are also used, depending on the application. Although many HPLC stationary phases are available and have been used for the analysis of surfactant formulations, none of these columns have been designed specifically for this application, nor are they capable of separating anionic, nonionic, and cationic surfactants in a single chromatographic run. Figure 1 shows the difference between a conventional C18 column and the Acclaim Surfactant column for the separation of a mixture of anionic and nonionic surfactants. The Acclaim Surfactant column provides excellent separation, whereas the C18 column fails to resolve all the surfactants under the same conditions.

Excellent Peak Shapes for Cationic Surfactants

Reversed-phase chromatography, using a C18 column, is often used for the separation of anionic surfactants. When analyzing cationic surfactants, however, it is often difficult to obtain sharp, symmetrical peaks due primarily to the presence of free silanols. The novel bonding chemistry of the Acclaim Surfactant phase allows for effective deactivation of free silanols toward positively charged cationic surfactants, resulting in excellent peak shapes, as shown in Figure 2. By comparison, a C18 column tested under similar conditions demonstrates an extended retention time and peak tailing.

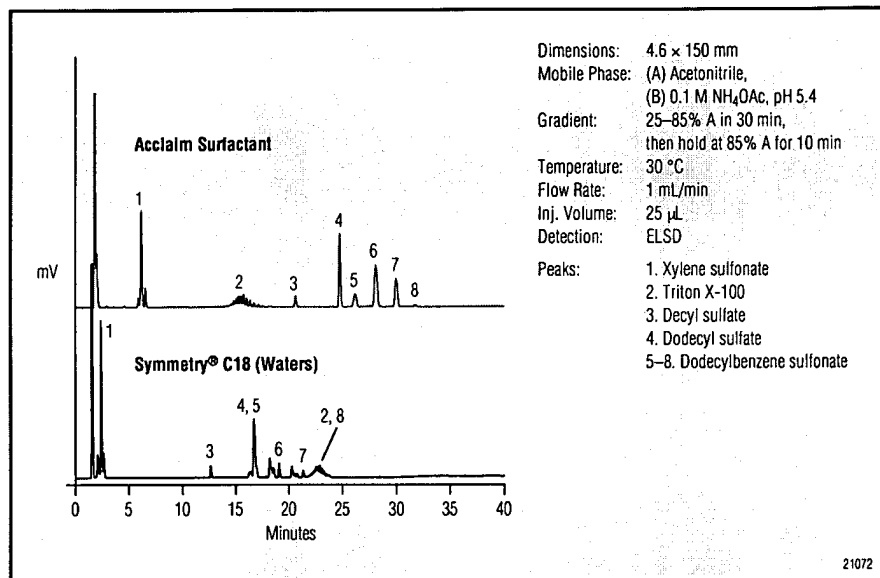


Figure 1. Separation of a mixture of surfactants showing remarkable selectivity.

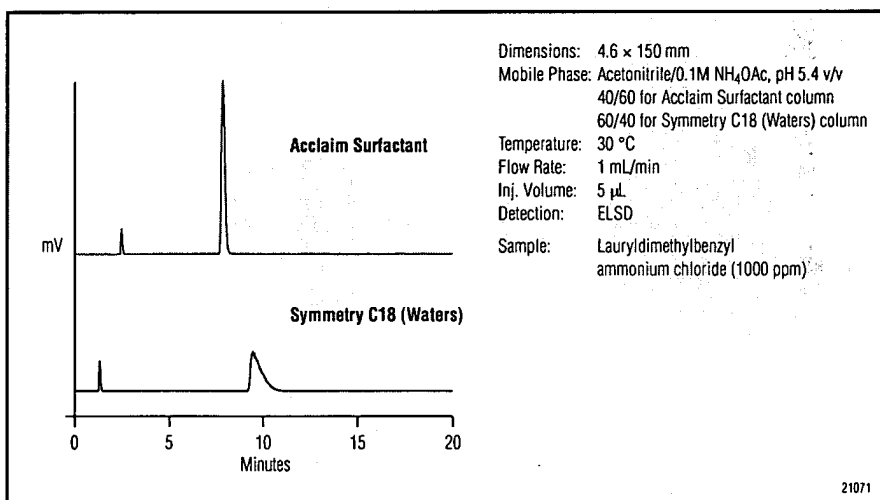


Figure 2. Analysis of a cationic surfactant showing excellent peak shape.

Improved Resolution for Ethoxylated Surfactants

As a consequence of its novel column chemistry, the Acclaim Surfactant column exhibits a unique polarity that provides significantly improved resolution for individual oligomers of ethoxylated surfactants compared with conventional C18. Figure 3 provides a comparison between the Acclaim Surfactant column and a conventional C18 for the characterization of Triton X-100. The Acclaim surfactant column exhibits significantly improved resolution between the oligomers.

Compatible with Highly Aqueous Mobile Phase Conditions

High-density C18 columns are often unsuitable for analyzing strongly hydrophilic hydrotropes, such as sodium naphthalene sulfonate and xylene sulfonate. The problem arises because these analyses require a highly aqueous mobile phase that often leads to undesirable "dewetting". As illustrated in Figure 4, the novel chemistry of Acclaim Surfactant column provides excellent resolution between isomers of xylene sulfonate, while under the same condition little or no retention is observed on the conventional C18 column.

Methods Compatible with Various Detectors (ELSD, UV, MS Conductivity Detection)

UV absorbance is the most popular detection method in HPLC, due to its ease of use and sensitivity (Figures 3–5 and 18–20). The drawback with this approach is that the analyte must have a chromophore to be detected and many surfactants do not.

Although refractive index (RI) detection is a universal detection method, capable of detecting all analytes, it is incompatible with gradient methods, exhibits low sensitivity, and thus is only used when other detection methods are not applicable.

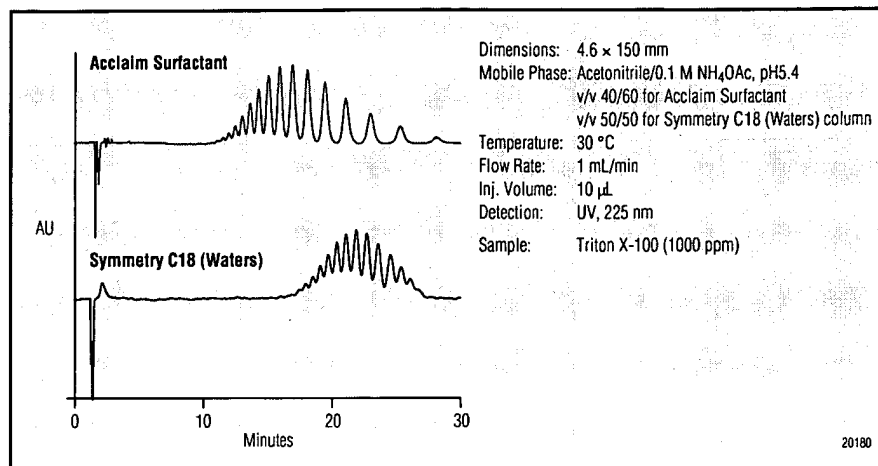


Figure 3. Improved resolution between oligomers in ethoxylated surfactants.

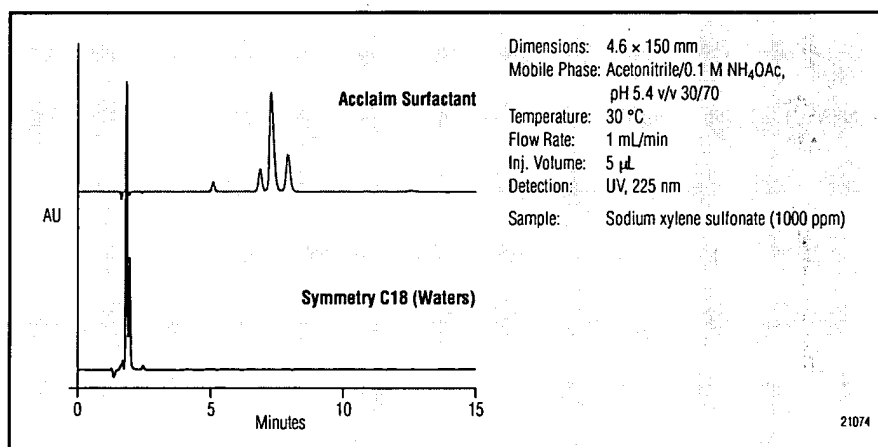


Figure 4. Analysis of a strongly hydrophilic hydrotrope.

Evaporative light-scattering detection (ELSD) is not only a universal detection method, but also is compatible with gradient methods and is far more sensitive than RI. In addition, methods developed with ELSD can be easily transferred to LC-ESI-MS applications with little or no modifications, because both detectors share the same mobile phase requirements (Figures 1, 2, 8, 11–14 and 16–22).

Mass spectrometry (MS) is an inherently sensitive and universal method and has become the widely accepted tool for characterization of organic compounds. The soft ionization techniques, such as electrospray ionization (ESI), have greatly increased

the applicability of MS detection to surfactant analysis. As shown in Figures 6, 10, and 15, the Acclaim Surfactant column can be used for the analysis of anionic, cationic, and nonionic surfactants using LC-ESI-MS and ammonium acetate eluents.

Suppressed conductivity detection can also be used for surfactant analysis and provides certain advantages for analyzing trace levels ionic surfactants in complex matrices. Figures 7 and 9 show the separation of various anionic and cationic surfactants on the Acclaim Surfactant column, using a borate buffer and acetic acid mobile phases, respectively.

Broad Range of Applications

Anionic Surfactants

Anionic surfactants account for 60% of surfactant use in the United States, where they are popular ingredients in detergent powders. This popularity arises because of their effectiveness compared with other surfactants in particulate soil removal, especially from natural fabrics, and because they are easily spray-dried.

Linear alkylbenzenesulfonates (LASs) are the most widely used surfactants, due to their low cost and rapid degradation under aerobic conditions. The synthesis of LAS typically leads to a mixture of positional isomers that results in a very complex sample matrix that can be a challenge to separate effectively by chromatography. To simplify quantitative analysis, isocratic conditions are often used to produce only single peaks for the same size homologues species. As shown in Figure 5, LAS can be separated on the Acclaim Surfactant column into simple, single peaks corresponding to a homologous series, whereas the Acclaim PA column gives more complex chromatograms.

Alkyl sulfates are the sulfuric acid esters of linear alcohols. They are frequently employed as additives in cosmetics and detergents. Figure 6 shows the analysis of lauryl sulfate, a major ingredient in shampoo, on an Acclaim Surfactant column using LC-ESI-MS and an ammonium acetate eluent.

Alkylether sulfates are prepared by adding oxyethylene groups to an alcohol that is then sulfated. Oxyethylation enhances water solubility and foaming, making these surfactants ideal components in shampoos and detergents. Figure 7 shows the analysis of laureth sulfate using suppressed conductivity detection.

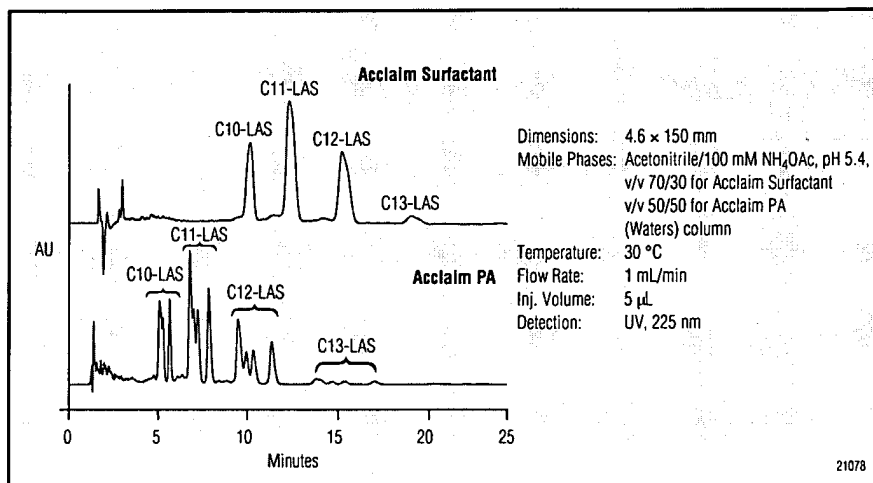


Figure 5. Analysis of sodium dodecylbenzene sulfonate (LAS).

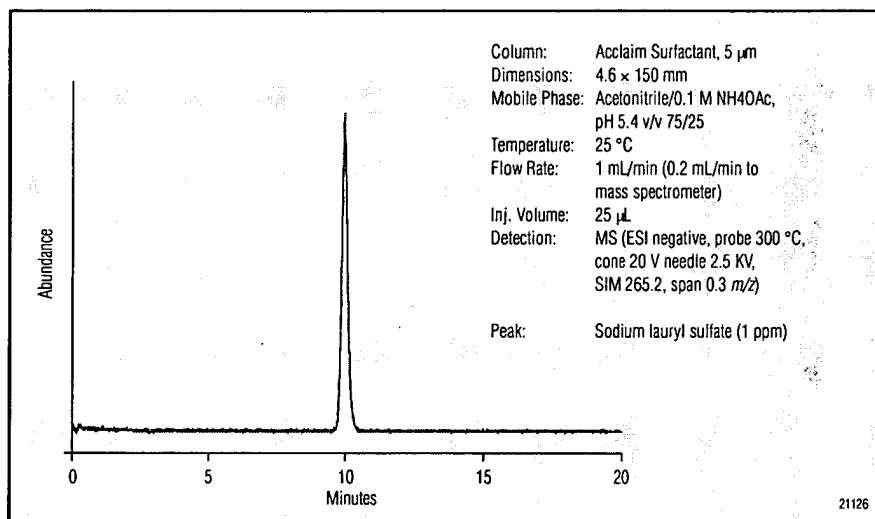


Figure 6. Analysis of sodium lauryl sulfate using LC-ESI-MS.

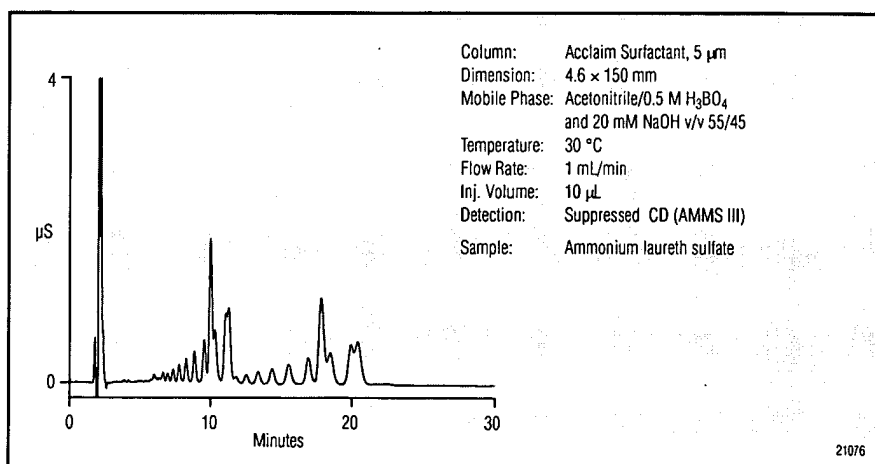


Figure 7. Analysis of ammonium laureth sulfate using conductivity detection.

Cationic Surfactants

Cationic surfactants are used as fabric softeners, corrosion inhibitors, and antimicrobial agents. The most popular cationic surfactants include alkyl quaternary ammonium salts, benzylalkylammonium salts, pyridinium salts, ester quats, ethoxylated quats, and quaternary imidazolium compounds.

Figures 8–12 present examples of chromatographic analysis using the Acclaim Surfactant column.

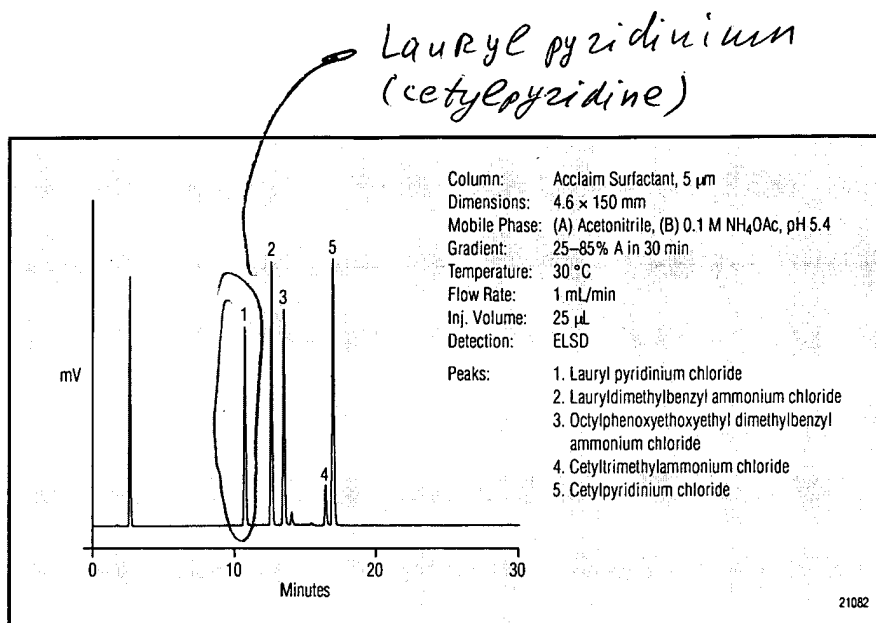


Figure 8. Separation of cationic surfactants using ELSD.

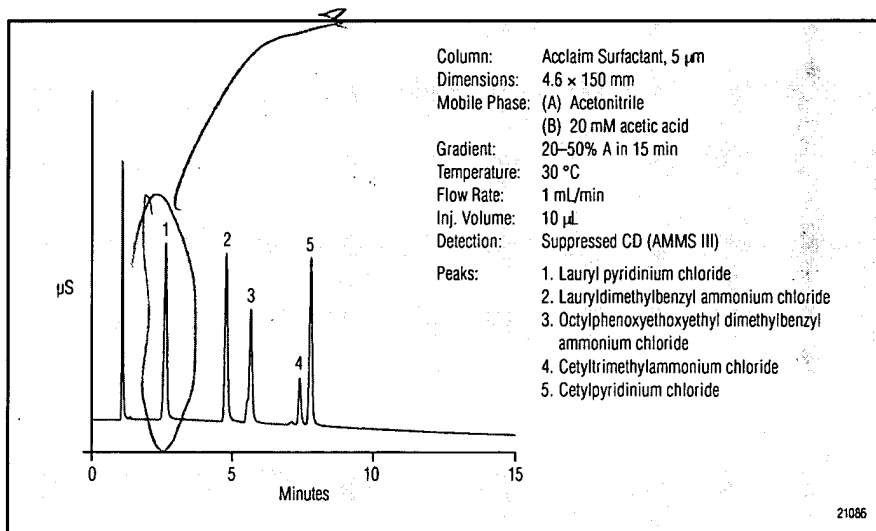


Figure 9. Separation of cationic surfactants with suppressed conductivity detection.

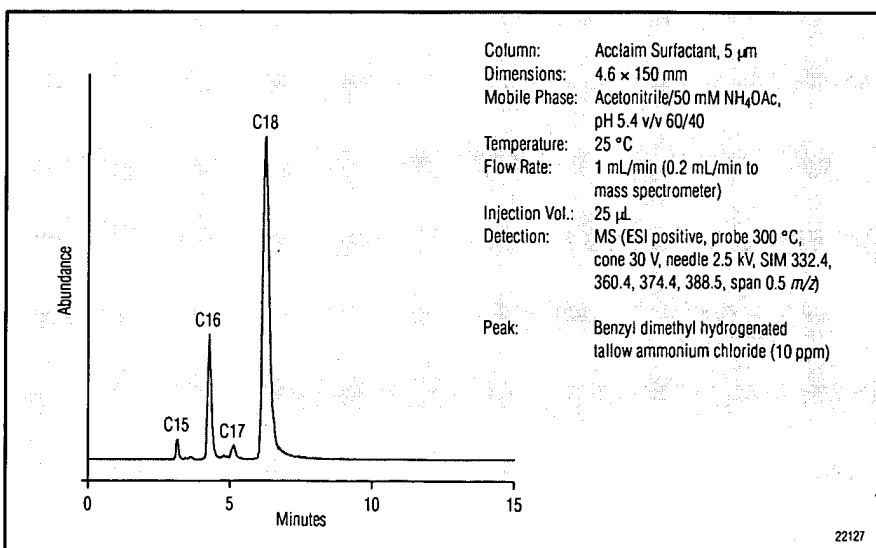


Figure 10. Analysis of quats using LC-ESI-MS.

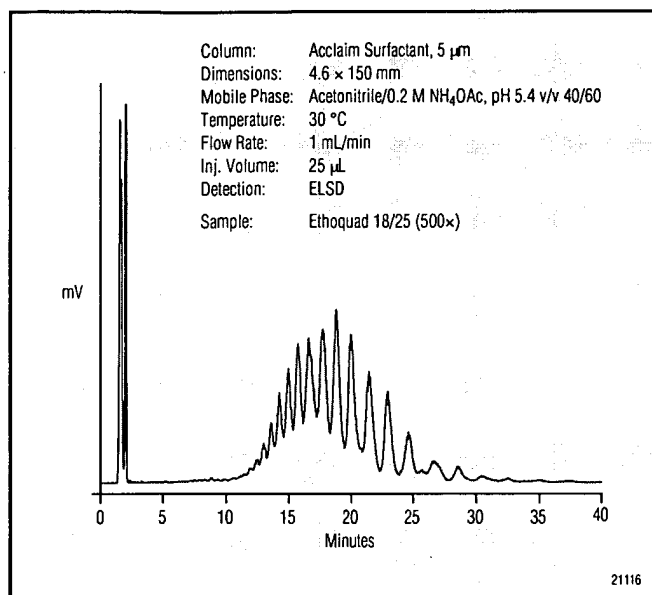


Figure 11. Separation of ethoxylated quats.

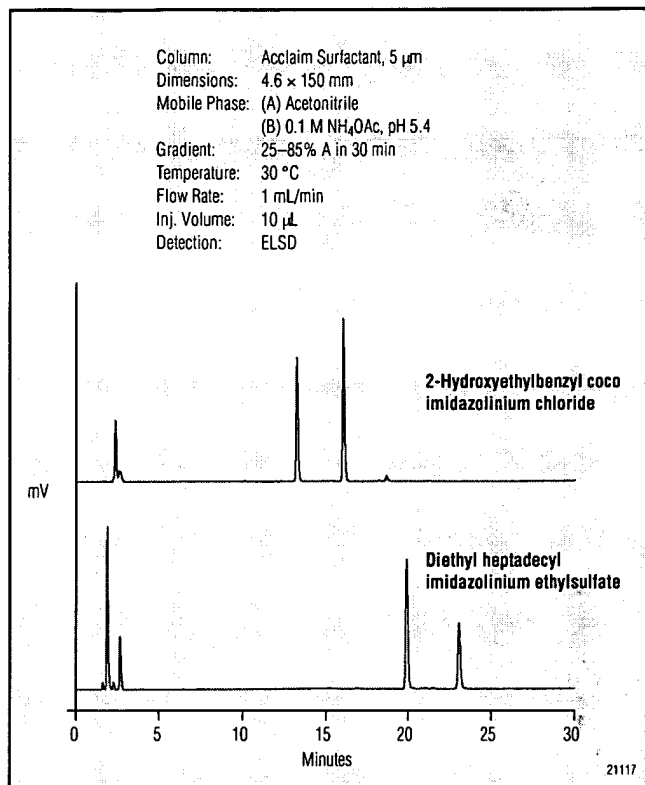


Figure 12. Separation of quaternary imidazolinium compounds.

Nonionic Surfactants

Nonionic surfactants account for about 40% of the worldwide consumption of surfactants. Most nonionic surfactants are considered low-foaming products, have good cold water solubility, and low critical micelle concentration. Their compatibility with cationic fabric softeners makes them preferable in certain formulations. Figures 13–16 show chromatographic analyses of three individual nonionic surfactants using the Acclaim Surfactant column.

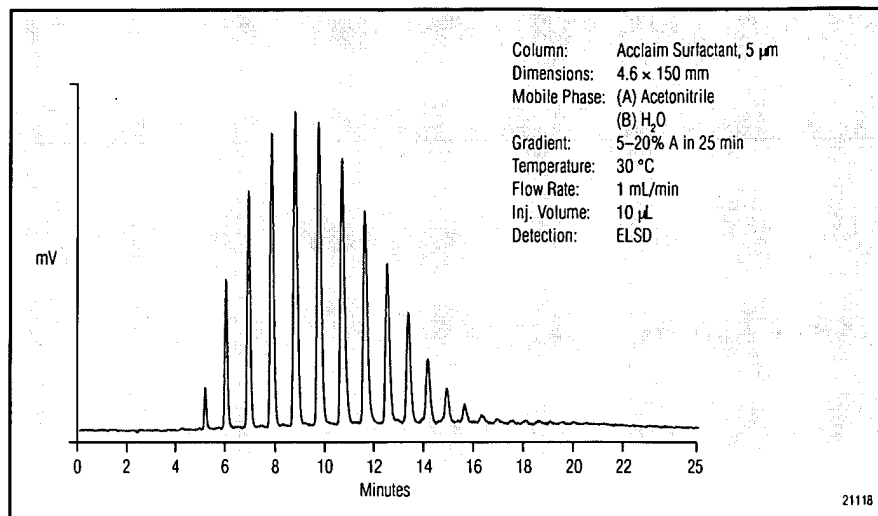


Figure 13. Analysis of PEG monoethyl ether (MW-550).

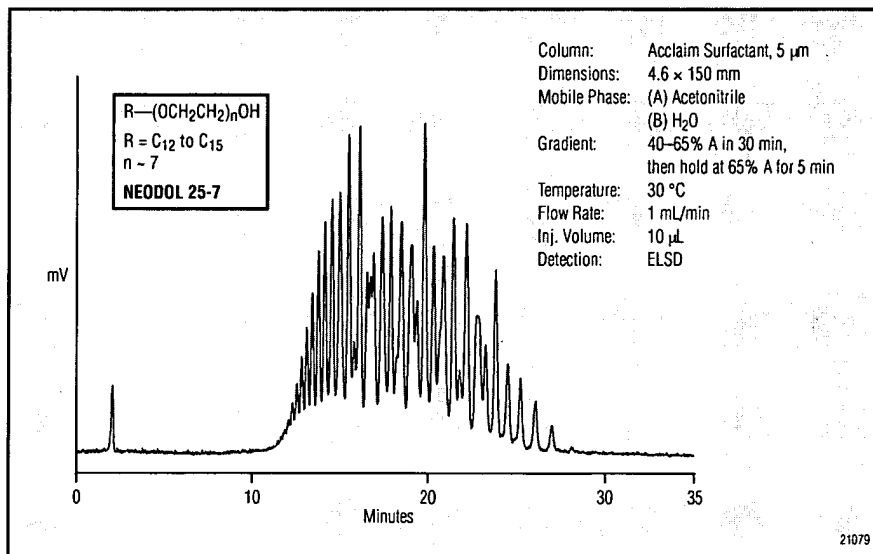


Figure 14. Analysis of NEODOL 25-7.

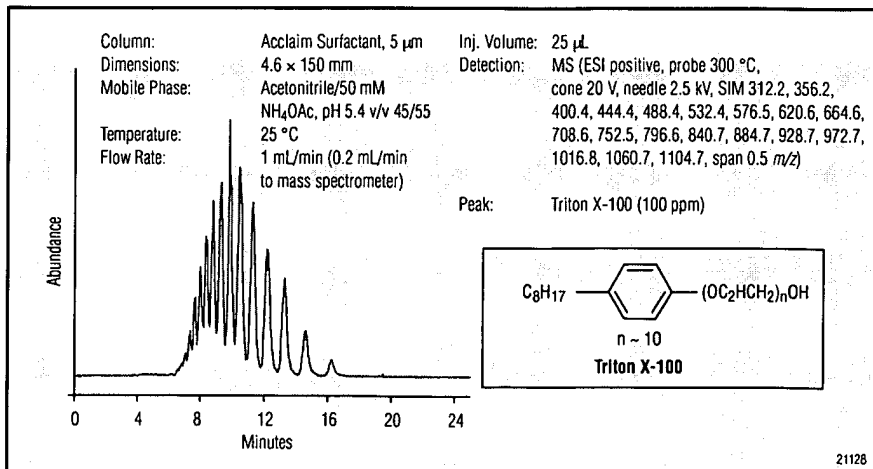


Figure 15. Analysis of Triton X-100 using LC-ESI-MS.

Polyethylene Glycols (PEGs)

Polyethylene glycols (PEGs) are often nonsurfactant impurities found in ethoxylated surfactants, typically in the range of 1–10%. The oligomer distribution is similar to, but broader than that of the surfactant. Figure 17 illustrates the exceptional resolution of the Acclaim Surfactant column for individual oligomers in various PEGs.

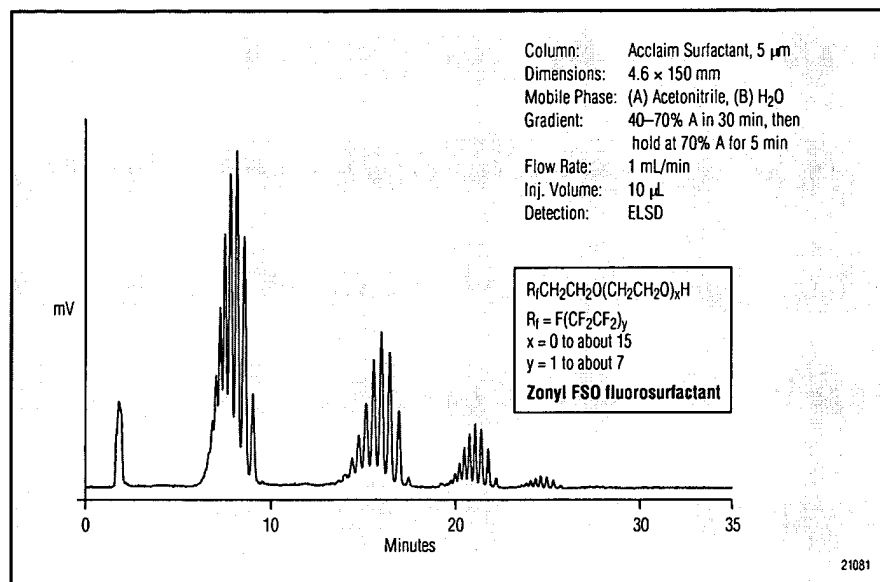


Figure 16. Analysis of ZONYL FSO fluorosurfactant.

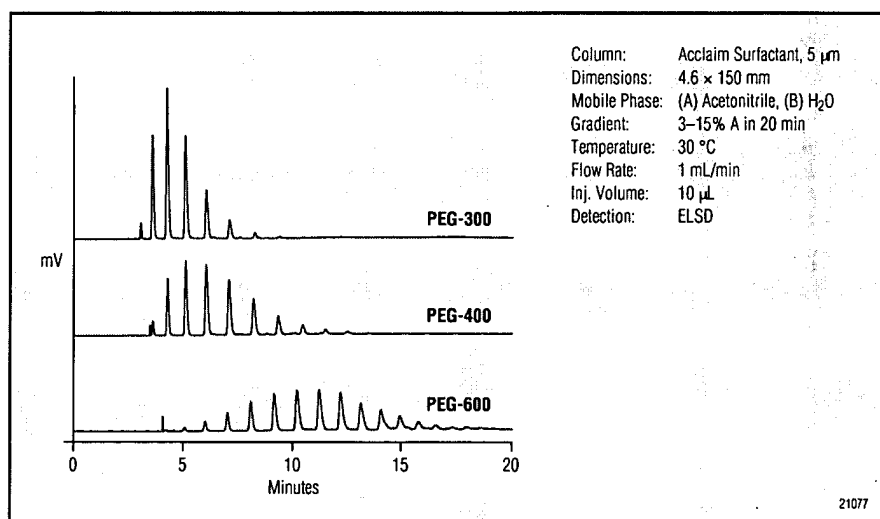


Figure 17. Separation of different polyethylene glycols.

Analysis of Surfactants in Consumer Products

Figures 18–22 demonstrate the applicability of the Acclaim Surfactant column for analyzing a variety of consumer products, such as shampoo, laundry detergent, dish washing liquid, mouthwash, and fabric softener.

Reproducible Manufacturing

To meet the exacting needs of our customers, each Acclaim Surfactant column is manufactured to stringent specifications to ensure column-to-column reproducibility. Each column is shipped with a lot validation sheet showing the test results and specifications for the lot of bonded silica packed into the column. In addition, each column is individually tested and shipped with an individual test chromatogram validating the column performance, with respect to selectivity, capacity, and efficiency.

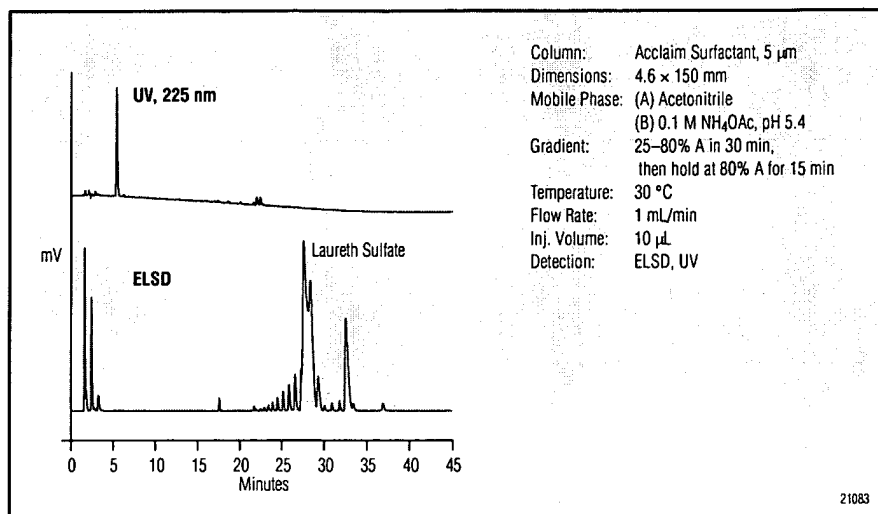


Figure 18. Analysis of a shampoo.

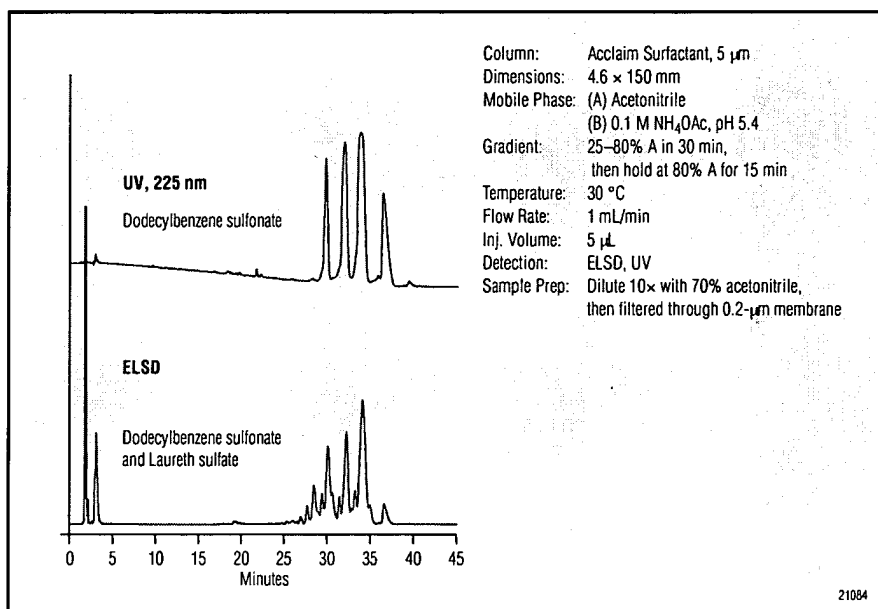


Figure 19. Analysis of a laundry washing detergent.

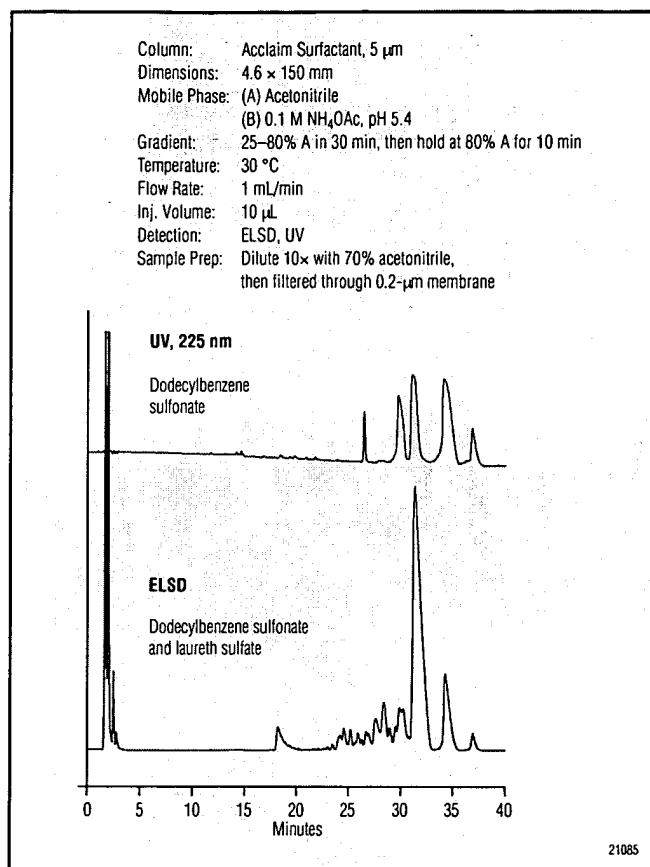


Figure 20. Analysis of a dishwashing liquid.

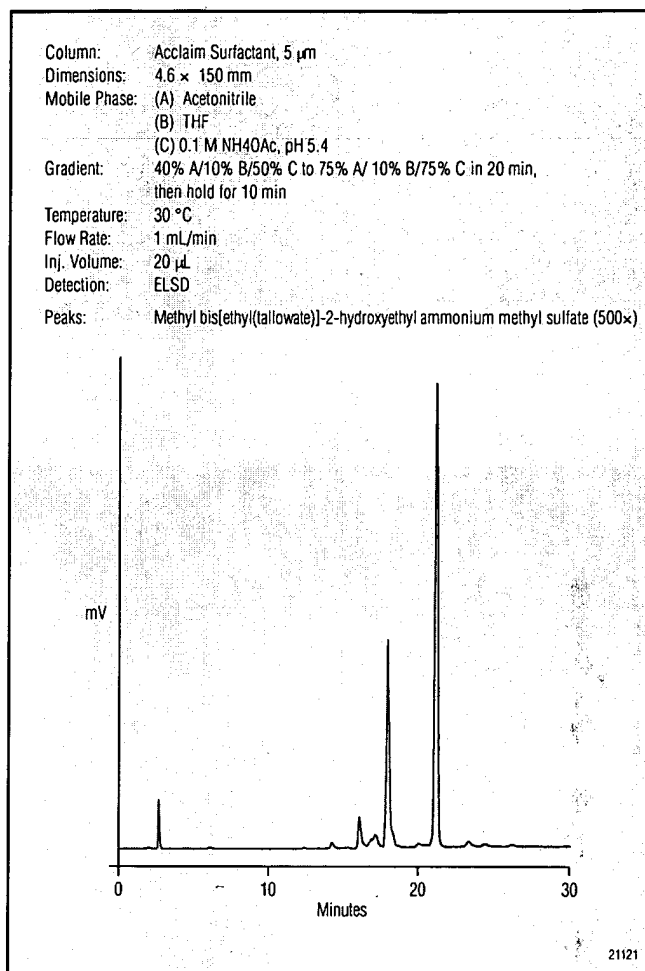


Figure 22. Analysis of a fabric softener.

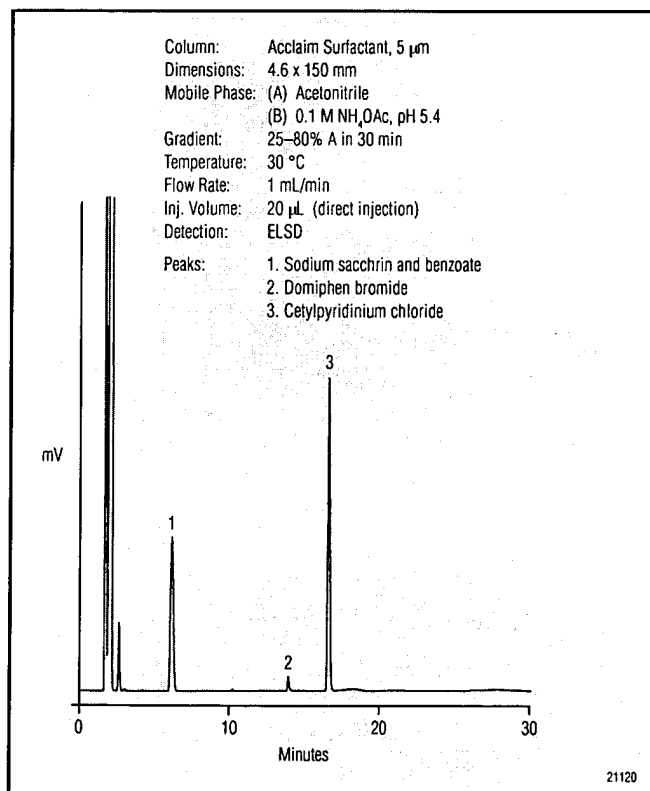


Figure 21. Analysis of a mouthwash.

SPECIFICATIONS

Starting Material:

Ultrapure silica

Particle Size:

5 μm

Particle Shape:

Spherical

Particle Size Distribution (40/90):

1.2

Total Carbon Content (%):

12%

Endcapped:

Yes

Metal Impurity (ppm) Na, Fe, Al:

<10.0

Pore Volume (mL/g):

0.9

Average Pore Diameter (\AA):

120

Surface Area (m^2/g):

300

pH range:

2.5–7.5

Temperature:

<60 $^{\circ}\text{C}$

ORDERING INFORMATION

To order in the U.S., call (800) 346-6390 or contact the Dionex Regional Office nearest you. Outside the U.S., order through your local Dionex office or distributor. Refer to the following part numbers.

Product Description	Part Number
Acclaim Surfactant Analytical Column (4.6 \times 150 mm)	063201
Acclaim Surfactant Analytical Column (4.6 \times 250 mm)	063203
Acclaim Surfactant Guard Cartridges (4.3 \times 10 mm), 2 ea	063215
Acclaim Guard Kit (holder and coupler)	059526
Acclaim SST Guard Cartridge Holder	059456
Guard to Analytical Column Coupler	059457

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Biggest Breakthrough in HPLC in 25 years!

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Author

Message

Supercritical

Posted: Wed Feb 16, 2005 3:50 am Post subject: Biggest Breakthrough in HPLC in 25 years! [quote](#)

Joined: 15 Nov 2004
Posts: 23

Primesep mixed mode columns. I've been working with these columns for 2 months and have developed separations on these mixed mode columns which are **impossible** on anything else available. This has been a dream column for years. Separating polar molecules, ionic molecules and neutrals in one method. Most of the time isocratic too! No one else has this technology that I know of. I'm certain all the big name column manufacturers are scrambling to produce copy-cat columns. Will be interesting to see. Anyone else been working with these columns? I think this is the biggest breakthrough in HPLC column technology in 25 years...that's how long I've been doing LC.

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SIELC_Tech

Posted: Wed Feb 16, 2005 4:48 am Post subject: [quote](#)

Joined: 31 Aug 2004
Posts: 240
Location: Prospect Heights, IL

Thank you Supercritical,

Is any way you can identify yourself to us, we would like to add you to our database - we can send you our monthly updates.

I believe that there are just a few people here who have used our column (I know at least four), but we are still trying to deliver our message.

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JA

Posted: Wed Feb 16, 2005 5:22 am Post subject:

quote

Joined: 10 Sep 2004
Posts: 96
Location: Manchester, UK

That's one of the boldest sales pitches I've read on here..

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profile

SIELC_Tech

Posted: Wed Feb 16, 2005 5:28 am Post subject:

quote

Joined: 31 Aug 2004
Posts: 240
Location: Prospect Heights, IL

JA,

I can assure you that nobody at SIELC posted the first message (on a Bible, under oath, on my helath or whatever...LOL) but you are free to express your opinion. Unfortunately Supercritical has no "personal" information with email address, which might show that he is not associated with our company.

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profile email www

Supercritical

Posted: Wed Feb 16, 2005 5:43 am Post subject: RE:

quote

Joined: 15 Nov 2004
Posts: 23

SEILC_Tech: Yes I am already on the mailing list! Thanks!

No I have nothing to do with the company that manufactures these columns. However, I have done LC for 25 years and I do believe this technology is the biggest breakthrough in HPLC columns that I have seen. This is my opinion, and I would like to hear other comments. As I stated, any column manufacturers in the know are definately pursuing this technology right now. This technology has overcome problems with traditional columns including those with embedded polar groups in regards to analyzing polar, ionic and neutral components in one quick easy method. This has been a limitation of HPLC in the pharmacuetical industry (probably the biggest end user of analytical and prep columns) ever since HPLC was invented. Time will tell. ☺

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profile

mtnshawn

Posted: Wed Feb 16, 2005 12:42 pm Post subject:

quote

Joined: 01 Oct 2004
Posts: 25

15 years of method development/validation in the Pharma business, and I also am a believer.

Had no success developing a small molecule method (8 component matrix) that would provide any resolution for my compound of interest. I spent approximately 1 month evaluating various columns/buffer on 2 LC's.


After a week of using the column, a method was developed. After a 2 week evaluation/qualification it was determined that the method was satisfactory for cGMP validation upon demonstration of batch to batch variability (or hopefullylack there of).


I do not work, nor do I know anyone who is associated with SIELC. Feel free to contact me @ scook@rxkinetix.com

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 [profile](#)  [email](#)

ananda

 Posted: Wed Feb 16, 2005 2:04 pm Post subject: Great News!

 [quote](#)

Joined: 23 Sep 2004
Posts: 33

Anyone of you have any experience analyzing peptides with this particular column and have acheived good separation of peptide impurities from its API?

Sounds like an intersting column but who is the manufacturer of this column?I can search but if you can tell me I can save my time:)

Thanks for the great news!


Ananda

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SIELC_Tech

 Posted: Wed Feb 16, 2005 3:46 pm Post subject:

 [quote](#)

Joined: 31 Aug 2004
Posts: 240
Location: Prospect Heights,
IL

Dear Ananda,

You can learn more about Primesep technology at

www.primesep.com

We will need more information on the size of your peptide (molecular weight, fragments, etc.) and your detection technique. You can check our method development guide for column and mobile phase selection:

<http://www.hplcmethoddevelopment.com/>


You can contact us at mail@sielc.com to discuss your particular application or check our library of methods (you can sort it by date, by compound or by application):


http://allsep.com/Applications_By_Column.php

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Bill Tindall

 Posted: Wed Feb 16, 2005 5:09 pm Post subject:

 [quote](#)

Joined: 30 Aug 2004
Posts: 134

I can understand being enthuisastic after solving a tough separation. But it seems a bit of an overstatement to imply that this is the first column that will separate "polar, ionic and neutrals", how ever polar and neutrals are defined in this context.

I can provide numerous examples of such separations on prior art columns. For

example, a great number of columns-Speherisorb ODS 2, Aquasep, and other hydrophilic columns- will separate aromatic difunctional acids along with some of their sulfonated analogs. While I never had the need to do so, esters of these acids would separate in the same run. (Anal.Chem. 63(1991)1251, for example.)


I am going to need more specific data before I agree that this column is a more significant advance than more inert silica, sterically hindered ligands, base stable packing and no doubt others .


Bill Tindall

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SIELC_Tech

 Posted: Wed Feb 16, 2005 5:35 pm Post subject:

 [quote](#)

Joined: 31 Aug 2004
Posts: 240
Location: Prospect Heights, IL

Bill,

I can understand your skepticism about this approach, If you (or anybody else) on this board have a tough separation we can try to develop the method and compare the results with any other column. The key in our technology is independent control for polar and hydrophobic compounds. You can enhance or suppress interaction with a simple modification of the mobile phase. Assuming that compounds have slight difference in hydrophobicity and polarity (ionization), by modifying both ion strength (and nature) and organic strength of the mobile phase, you can "pull apart" a lot of critical pairs and very often with isocratic conditions. The advantage of this approach is obvious in quantitation and preparative chromatography was by modification of two parameters of the mobile phase you can control the elution order of compounds.

Check this power point presentation for a few good examples:

<http://allsep.com/brochures/SeparationDifferentCompounds.pdf>

Another advantage of this approach is that you can use one column in several modes: RP, ion-exchange, ion-exclusion, normal phase, HILIC and mixed-mode. Here is another link with examples:

<http://allsep.com/brochures/UniversalStationaryPhase.pdf>

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Uwe Neue

 Posted: Thu Feb 17, 2005 9:01 pm Post subject:

 [quote](#)

Joined: 30 Aug 2004
Posts: 682

I really would like to understand the origin of the enthusiasm. What is easier? What is more complicated? Does the level of complication in the interaction mechanism present a problem or an opportunity?

When I did hands-on applications work myself (in the last century), I worked with a C18 column full of silanols that was so bad that barely anybody wanted to touch it. However, I was very familiar with it and I could get wonderful results with this column independent of the sample or the pH. To some degree, this column was similar to these mixed-mode packings. Many times, I could do separations that were difficult on a better C18. Are these mixed-mode things similar?

[Back to top](#)[!\[\]\(9dfdaff1d86ba3c1f8353b4d1b61b8c5_img.jpg\) profile](#) [!\[\]\(bcef2083a617d3f771f1bcdf2f97158d_img.jpg\) email](#)**Supercritical**

Posted: Fri Feb 18, 2005 4:52 am Post subject:

[!\[\]\(642aa997563f9a325b310230bb5078b7_img.jpg\) quote](#)

Joined: 15 Nov 2004
Posts: 23

Quote:

What is easier? What is more complicated? Does the level of complication in the interaction mechanism present a problem or an opportunity?

Doesn't matter to me. Primesep columns can do what no other column can do. Read what I said above. The column interactions are completely predictable if you understand the column chemistry and the chemistry of your sample components. I have found that I have much more control over the separations with these columns....a good thing for all chromatographers.

I've fought with difficult separations using ion pair reagents, tandem ion exchange/ RP columns, multiple methods, etc... These column can handle all that stuff & more. I do a lot of prep too, I can't mess around with Ion pair. Plus ion-pair goofs up the MS. Goodbye PIC reagents!

Look through the posts on this site and, and see all the questions about ion pair, organic acids, IEC, bases & amines, etc...!!! Lots of problems here for chromatographers. My suggestion is, if you got these problems, take a look at the Primesep line. I know that this may sound like a sales pitch, but it is not. This is a forum for chromatographers to spread knowledge and help one another, and that is my intent.

[Back to top](#)[!\[\]\(f219cfc00b8db0cd1a81ae1fc9afaf28_img.jpg\) profile](#)**ananda**

Posted: Fri Feb 18, 2005 10:07 am Post subject: Thanks!

[!\[\]\(df47d6bec273bbb8b349135fff3a20f7_img.jpg\) quote](#)

Joined: 23 Sep 2004
Posts: 33

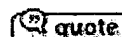
SIELC:

Thanks for the information. I will keep this information handy, even the page of everybodys comments here. for future reference. So far most of our peptide methods have been validated. Anyway, thanks again for the information.

Ananda

[Back to top](#)[!\[\]\(9cfd7b8995754ae2aef7ec59dba55501_img.jpg\) profile](#)**Bill Tindall**

Posted: Fri Feb 18, 2005 3:46 pm Post subject:



Joined: 30 Aug 2004
Posts: 134

The purpose of this note is to be constructive, not critical.....

Successful scientists are by nature skeptical folk. So a headline like what started this thread invites a skeptical response unless the claims are supported by data or explanation. There has been little of either.

As I have pointed out, this is certainly not the only column that can separate the classes of compounds claimed. So that claim by itself did nothing to diminish my skepticism. It was stated, however, the retention of these classes can be independently(easily, predictably???) controlled. That statement began to kindle some interest. If someone would tell us how it works, how its chemistry is different, how retention of compound classes is independently affected and specifically a separation it can do easily where traditional columns have failed, then I would see for myself what a "breakthrough" this technology might be. I think that forum participants would consider such information "science" not "advertising" and hence it would be acceptable (speaking for myself only).

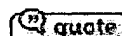
Bill Tindall



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SIELC_Tech

Posted: Fri Feb 18, 2005 4:24 pm Post subject:



Joined: 31 Aug 2004
Posts: 240
Location: Prospect Heights,
IL

Bill,

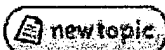
I might try to explain mechanism of retention and elution control but it might take too much space on this thread. I think it is more productive that you try to download all our brochures, newsletters and posters. If you would like we can add you to our distribution list and in this case you will receive all updates. Check the following link for Primesep literature:

http://allsep.com/Brochures_Home.php

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Vlad Orlovsky <vvorlo@gmail.com>

RE: Primesep Publication

1 message

Vlad Orlovsky <vlad.orlovsky@sielc.com>**Thu, Nov 10, 2005 at 7:15 AM**

Reply-To: vlad.orlovsky@sielc.com

To: Yury Z <zelechonok@gmail.com>

----- Forwarded message -----

From: **choechee@dso.org.sg** <choechee@dso.org.sg>

Date: Nov 10, 2005 2:30 AM

Subject: RE: Primesep Columns

To: Vlad Orlovsky <vlad.orlovsky@sielc.com>

Dear Vlad

I am interested in the Primesep AB column. I would like to ask the following questions:

1) I have a sample mixture of ethanolamine and different types of phosphoric acid and phosphonic acid in water and organic solvent. Do you think that the Primesep AB column will be an ideal column to separate and retain these totally different compounds in one single run? I am thinking of buying it to try out.

2) I have not been very successful in direct analysis of caffeine in plasma by C18 column after deproteination by ACN. I was successful to see your publication. The data SIELC shown on the website belong to human or animal plasma? How many injection before the column was affected by the protein from the plasma?

Lastly, I would like to inform you that my publication on the primesep 100 has been accepted by Journal of chromatography A. The compounds analysed were nitrogen mustards and its degradation products. Thank you once again for the 'magic' column.

Thanks and regards
Hoe Chee

--
Vlad Orlovsky
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www.sielc.com

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